MORPHOLOGICAL STUDIES OF IRRADIATION EFFECT ON SOME CROP PLANTS

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This is to certify that the dissertation entitled "Morphological Studies of Irradiation Effect on some Crop Plants" is a bonafide work carried on by the candidate Mr. S.A. Chaghtai, under the supervision of the undersigned. It is to be submitted in partial fulfilment of the requirements for the award of the degree of Master of Philosophy in Botany.

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C O N T E N T S

INTRODUCTION

Ever since the discovery of X-rays and radioactivity, the ability of the high energy practices to penetrate the living systems has excited a great and sustained interest in biologists. Earlier data which led to the formulation of quantitative hypotheses in radiobiology was principally concerned with such biological entities as bacteria, viruses and enzymatic systems which neither have a nervous system nor a complicated physiology. Few attempts have been made to use higher plants or animals for testing quantitative hypotheses, desirable though it would be on many grounds to obtain further insight into the action of radiation on higher organisms. Higher plants provide exceptionally favourable material for many basic studies in radiobiology. One aspect of the usefulness of plant systems involves the feasibility of experimentally separating growth, cell division and senescence etc. from one another. Plant development represents a regular pattern of growth and differentiation processes including numerous correlative phenomena. Exposure of green plants to ionizing radiations may upset this balance and induce the morphological changes in cells. Such changes are usually reflected as morphogenetic abnormalities.

In recent years ionizing radiations have been greatly exploited in understanding various fundamental problems of life processes and in improving crops through

mutations and subsequent breeding of these mutants. The ability of ionizing radiations to speed up the frequency of teratological changes has been utilized to throw light on the morphological nature of the organ. Radiation, therefore, may be considered as a valuable tool for developmental analysis, particularly where many events in growth are obscured (or not observed) because of the time factor.

A considerable amount of literature is presently available on the effects of ionizing radiations on plants (Johnson, 1936; Sparrow, 1951; Gunckel and Sparrow, 1954; Bacq and Alexander, 1961; Sparrow and Evans, 1961; Bari, 1971; Nayar, 1971; Sinha and Godward, 1972; Gupta, 1976; Nair and Nair, 1977; Chaghtai et al., 1978; Bandyapadhyay and Bose, 1979). As pointed out by Gunckel (1957, 1965) the response elicited depends upon the species, its age, physiological conditions, radiosensitivity, dose rate and various environmental conditions. Gunckel (1957) has emphatically suggested that the results from one species or variety should not be expected in different plants or even from different stages of develop-ment in the same plant.

Studies pertaining to the effect of radiations on cultivated plants have been mostly confined to barely, wheat, broad bean and peas. Most of the important oil-yieldings and proteinaceous field crops which have not yet received as much investigating attention as they

deserve are: Sunflower, Sesame, Soybean, Lentil, some Madhya Pradesh cultivars of flax etc.

Studies on the effect of Chronic and acute irradiation on seed-germination, survival of seedlings, morphological variations and yield in these crop plants are extremely meagre and provide scope for further investigations.

REVIEW OF LITERATURE

A-GENERAL ACCOUNT

General morphological effects following irradiation have been described recently by many authors (Ehrenberg et al 1953; D'Amoto, 1957; Preadcencu Al, et al., 1961; Bari, 1971) and have been reviewed earlier for acute X-irradiation (Johnson, 1936) and more recently for Chronic gamma-irradiation (Sparrow, 1951; Gunckel, and Sparrow, 1954; Gunckel, 1957; Beard, 1970 and 1971; Yadav and Dalal, 1971; Lobana et al, 1973).

Sparrow and Evans (1961) have published bibliography on the effects of ionizing radiations on plants from 1896 through 1955. Bacq and Alexander (1961) have done commendable job in their books entitled "Fundamentals of Radiology" and "Cellular Radiology" respectively. Proceedings of series of # symposium on the effects of ionizing radiations on seeds, published by International Atomic Energy Agency, Vienna (1961), provides a good deal of information on the subject.

B- SEED GERMINATION

Effects of X-rays and gamma-rays on seed germination in higher plants have been studied by several workers such as Johnson (1928), May and Rosey (1958), Gustafson and Simak (1958), Bora(1961) Bowen and Thick (1961), Harring et al (1964), Rai (1971), Bari (1971), Amanthaswamy et al (1971),

Chopra (1972), Ravindranath (1974), Chaghtai et al (1978 a,b,c), Abidi and Ghouse (1979), Chaghtai and Prasad (1979 a,b).

Rajan (1969) reported certain lower doses of ionizing radiations to be stimulatory for seed-germination in Sesamum while delayed germination has been reported in the gamma-irradiated dry seeds of Corchorus sp. and Phaseolus vulgaris. Bari (1971) found no clear difference in the germination of Linum seeds given acute doses from 10 KR to 250 KR of gamma rays.

Raghuvanshi and Singh (1977) while studying the effects of gamma-rays and some chemical mutagens on seed germination and seedling morphology of Capsicum annuum L. observed that both physical as well as chemical mutagens affected the percentage of seed-germination and a slight shift in most of the treatments but 0.03% DDA induced earliness. Gamma-rays alone or in combination with DMS or DMSO deferred in the day of initiation. The effect was found to be a synergistic one with gamma-rays + DMS. The effect of combination treatments on the frequency of germination was almost intermediate to that of individual mutagens. Maximum delay of 12-16 days in initiation of emergence was recorded with higher doses of gamma-rays (40 KR).

Recently Abidi and Ghouse (1979) while studying the effect of acute gamma-irradiation on the seed germination of Linum usitatissimum L. Var. Neelam observed that lower doses like 25 KR or 50 KR effectively promote the germination process to the extent of about 20% over the control when applied to the seeds in dry condition, while higher doses like 100, 125 or 150 KR bring about a gradual decline in the process in a positive manner. In case of gamma-irradiated seeds of Capsicum annuum Var. K_2, Chaghtai and Prasad (1979 a) observed an inverse linear correlation between the germination percentage and dose rate. Almost similar results were obtained with Var. K_1 (Chaghtai and Prasad, 1979 b).

Caldecott (1954) described an inverse relationship between water content of seeds and their sensitivity to X-Rays. Later working with barley seeds, he found that sensitivity decreased as the water content increased from 4% to 8%. Further addition of water resulted in no additional modification of sensitivity. Gustafsson and Simak (1958) also found an inverse correlation between water content and X-Ray sensitivity in pine seeds. Immature seeds were found more sensitive than fully matured ones. At equal doses, X-Rays had a stronger depressive effect than gamma-rays. Chaghtai et al., (1978 a and b) reported a notable difference in germination response of the dry and presoaked gamma-irradiated seeds of Phaseolus mungo and Lens esculenta. For P. mungo similar results were also reported by Siddiqui et al., (1979).

Ehrenberg et al.,(1953) reported that a change in Oxygen pressure at the time of irradiation has profound effect on physiological processes, behaviours and responsds examined in barley seeds.

Once the germination process is initiated, radiation sensitivity is altered. Thus the response of germinating seeds as well as of young seedlings towards irradiation is markedly different as compared to the unactivated or dormant seeds. Nybom (1956) found that a number of seedlings reacted in the same manner whether the radiation was continuous or intermittent during 12 hrs. either day or night. On the other hand Biebl (1959) found a notably higher sensitivity as measured by the depression of subsequent organ growth when he X-rayed germinating wheat grains on the second day of germination (since the Soaking of seeds).

It has been recognised that temperature, water content, oxygen tension, radio protective substances in the seed and the type of ionizing radiation may all affect seed-germination as well as growth of seedlings (Nybom et al., 1952; Caldecott, 1955 a.b.c.; Conger and Randolph, 1959).

In addition to the factors listed above, radiosensitivity of a plant has also been reported to depend on its Karyotype. Plants with comparatively smaller chromosomes tend to be less sensitive than those with larger ones. Within the same genus normal diploids have been found more sensitive towards radiations than the polyploid species (Sparrow and Evans, 1961).

Recently X-rays have been reported to stimulate germination of some highly dormant wild papilionaceous seeds (Chaghtai et al, 1978 c)

C-SEEDLING SURVIVAL

Reduced seedling survival as an after effect of irradiation has been reported by Caldecott (1955 b) and Rai (1971). Bari (1971) also found that survival of flax plants was very low at 150 KR and 200 KR doses of gamma-rays and there was practically no survival at 250KR.

D- GROWTH HABIT RESPONSES

Sparrow (1951) noticed an increase in plant height of Antirrhinum majus at exposure rates above 125 R/day, reaching to maximum at 230 R/day. Gunckel and Sparrow (1954) reported simdlar stimulation of growth and notable increase in plant height, in Antirrhinum and Nicotiana spp. when subjected to moderate exposure of chronic gamma-irradiation. Ehrenberg et al., (1954) observed growth stimulation in Vicia faba at daily exposure range of 16-28 R of gamma-irradiation. Studying the effect of Chronic gamma-irradiation, Nikaelson and Aastveit (1957) observed a considerable increase in plant height at exposures ranging from 25 to 34 R/day in barley. Similar behaviour was recorded by sparrow and Evans (1961) De Nettan Court and Contaat (1966) found marked increase in plant height of Lycopersicum sp. under continuous radiation exposures. Davies (1968) recorded growth stimulation due to irradiation in a wide variety of higher plants. Bostrack and Sparrow

(1970) observed a significant increase in height at exposure rates of 1-2 R/day in Pinus Strobus.

On the contrary there are equally numerous reports regarding the inhibition of seedling growth and height of plants under the influence of irradiation. Johnson(1936) held the opinion that injury and growth are the most common effects following X-ray exposure. Sparrow (1951) reported a general decrease in plant hight with every increase in dose level. D'Amato (1957) in his studies on the effect of Chronic gamma irradiation in flax did not find any growth stimulation except in the early stages of plant growth at 375 and 425 R/day. It was, however, not ascertained as to whether the difference was due to some stimulatory action of radiation or to some differences in soil condition. Since then growth inhibition by ionizing radiations has been reported in various plants by many workers (Gunckel and Sparrow, 1961); Dumanovic and Ehrenberg, 1965; Davis, 1968; Chauhan, 1969; Rai, 1971 and Chopra, 1972). Beri (1971), during his studies on acute irradiation on flax, found that the plant height at maturity decreased with increasing exposures. While on the other hand, in the chronically exposed plants of Flax he noticed that plant height at maturity increased gradually as the daily exposure rate increased from 100 to 600 R/ followed by a sharp decline thereafter. At 1000 R the plant height was less than that of the non irradiated plants. Maximum average height was observed in plants subjected to a daily exposure of 600 R.

Different workers hold different opinion regarding the phenomenon of stunted growth resulting from irradiation. Important suggestions are : (1) uneven damage to meristematic cells due to genetic injuries (Gray and Scholes, 1951; Lea, 1955), (2) Chromosomal damages or inhibition of cell division (Sparrow and Evans, 1961; Conger and Stevenson, 1969), (3) marked decrease in the auxin level following irradiation (Moh and Smith, 1951; Gordon, 1954, 1957), (4) marked effect on auxin synthesis (Gunckel and Sparrow, 1961) and (5) effect on respiratory enzymes (Bjornseth et al., 1957).

Quastler et al. (1952) held both irradiation and physiological disorders responsible for the stunted growth.

E- STEM MODIFICATIONS

X-rays induced stem abnormalities were observed by Johnson (1936) while Gunckel et al. (1953) reported similar abnormalities induced by gamma rays in case of Tradescantia where the young stem exhibited distinct swelling at the base of upper internodes accompanied with slight twisting of the branches but the effect was found temporary and short lived as the normal pattern was soon restored with the advancement of growth.

Common responses of stems to irradiations are dwarfing and excessive branching from abnormal development of axillary or adventitious buds. Irradiated stems may also show swelling and twisting of internodes, fasciations dichotomy, altered phyllotaxy, stem lesions, an increase

in the amount of vascular tissue and formation of stem tumors (Gunckel and Sparrow, 1954).

Fasciation and similar growth abnormalities in stem and leaves have been observed following irradiation with X-rays, (Irving, 1940) or gamma-rays (Gunckel and Sparrow, 1954; D'Amato, 1957). Ionizing radiations and growth regulators have been shown to bring about identical fasciation in plants (Gorter, 1965). In higher plants particularly the induction of fasciation following irradiation is of most frequent occurrence.

Induction of dichotomies and altered phyllotaxy have also been demonstrated in certain cases following exposure to gamma-rays and thermal neutrons (Johnson, 1933, 1948; Irvine, 1940; Gunckel, 1965).

Fasciation of flower stalks due to X-irradiation has been observed in sunflower (Johnson 1926), flax (D'Amato, 1957). In irradiated and fasciated stems of Linum, D'Amato (1957) reported the xylem ring to be thinner than in control and there was greater variability of fibre-cell diameter and wall thickness although fasciation did not affect fibre length. Abidi et al. (1979 a) and Ghouse et al. (1980) while working on an Indian oil Yielding variety T397 of flax observed that gamma-irradiation seriously affects the development of primary body as well as secondary structures in a direct correlation to the dose rate. The greater was the level of radiation intensity, the more was the damage caused to the water conducting system as well as to the mechanical tissues of the plant.

Sparrow (1951) and Sparrow and Evans (1961) reported development of adventitious meristems and tumors on the irradiated stems of Nicotiana glauca with higher dose rates or longer exposure at any one dose. The increase in number of plants with tumors was accompanied by an increase in the amount of tumor per plant.

F- LEAF ABNORMALITIES

Reduction of leaf blade, twisting of the entire leaf or leaflets and fusion of leaf parts have been reported as common features in irradiated Helianthus seedlings (Johnson, 1926) and tomato plants (Johnson, 1931). Similar results for a number of plants were reported by Goodspeed, (1929), Morgon (1931) Haskins and Moore (1935). Leaf thickening has been shown to increase with dose rate (Gunckel and Sparrow, 1954). Response of the leaves of Antirrhinum majus was studied at 17 dosages of gamma-rays ranging from 0.5 to 600 r/day. A progressive thickening of the leaves was observed above 240 r dose which increased to three times that of controls at 600 r/day and the leaves had a leathery texture. Beibl (1959) also observed similar effects of Gamma-irradiation on leaves of higher plants.

Abnormalities pertaining to leaf colour, form and texture have been found to be induced by several

workers in many plants following suitable doses of ionizing radiations. Leaves show a wide range of responses like the appearance of a mosaic or over all colour changes, dwarfing, premature abscission, increased pubescence, changes in leaf form and texture accompanied with irregular blade development such as distorted venation, puckerring between the veins, fusions, thickening, development of double leaf-blades and formation of tumors (Johnson, 1936; Gunckel and Sparrow, 1961; Gunckel, 1965; Bajaj et a 1970; Chopra 1972; Seetharam and Srinivasachar, 1972; Bandyopadhyay and Bose, 1979).

G- FLORAL TERATOLOGY

In addition to stimulation of growth in length of floral stalks and abnormal thickening including fasciation, irradiated plants have been found to show: (1) abnormal vegetative growth in floral positions (Sparrow, 1951; Gunckel et al., 1953 a,b) (2) delayed or reduced flowering (3) colour changes and other somatic mutations, (4) high degree of sterility, (5) early abscission, (6) modification in form and number of floral parts especially those of petals and stamens (Gunckel et al., 1953 b). Most of these effects are discussed in detail in a review paper by Gunckel and Sparrow (1954).

Irradiated sunflower plants have been reported to produce fasciated flowers (Johnson, 1926) while production of multiple flowers or abscission of flower buds (depending upon the stage of development at the time of irradiation) have been observed in case of tomato (Johnson 1931). Hoskins and Moore (1935) reported premature flowering in grape fruit plants from X-irradiated seeds. Johnson (1936) reported delayed and reduced flowering in a number of plants. Early blooming in a group of irradiated Kalanchoe plants has also been observed (Johnson, 1938). The inflorescence of Tradescantia paludosa, receiving 20-25 R dose/day for 8 weeks proliferated into a globose head by the formation of leaf like structures and modified flowers. Removed from leaf like structures and modifed flowers. Removed from radiation source and allowed a recovery period, these heads developed a large number of apparently normal vegetative shoots (Gunckel et al., 1953 a). Abscission of flower buds in tomato, tobacco, snapdragon and several other plants growing in gamma-field has been reported by Gunckel and Sparrow (1954). Stimulated flowering has been demonstrated in Tradescantia paludosa (Gunckel et al., 1953 b), Nicotiana rustica (Gunckel and Sparrow, 1954, Impatiens sultani (Gunckel, 1957). It has been found that with the increasing doses of chronic gamma rays flowering is generally retarded (Gunckel, 1965). Bari(1971) reported delayed flowering in Linum with prolonged radiation ----

exposure and at 100 r/day flowering was initiated about a month later as compared to the control.

Occurance of colour chimeras in the flowers after X-ray treatment was observed by Moore and Huskins (1935) Appearance of similar chimeras has been reported in several plants by Sparrow (1951)

Johnson (1936) observed sterility to be a common by-product of X-ray treatment. Sterility was quite common at high dosages in chronically irradiated plants, particularly in those flowers which were premature in flowering (Gunckel et al., 1953 a; Gunckel and Sparrow, 1954). These findings have been reaffirmed by Kumar (1971), Kumar and Singh, (1972), Rogers and Xavier (1972). In Linum usitatissinum L.Var. T397, Abidi et al., (1979 b) found that gamma rays induced male sterility in a direct correlation to the dose rate. The progeny turned out to be almost completely male sterile at 150 K rad while it was 50% at 25 K rad dose.

The most frequent modifications in floral development after irradiation are in the form and number of flower parts. Abnormalities commonly include modified petal lobes, increase or decrease in the number and size of petals or inhibition (Johnson, 1936; Gunckel et al., 1953 a; Horlan et al., 1973).

Bordy (1953) showed a wide range of abnormalities in tomato including multiple, fasciated or single, open or closed ovaries, multiple or fused styles, extra stamen traces, formation of meristematic areas on anther or filament which gave rise to embryo sacs and microspore as well as megaspore mother cells in adjacent locules or even in the same locule of an anther.

Bari (1971) and Badwal et al., (1972) reported abnormalities pertaining to seed-yield and seed-morphology in irradiated progeny of flax. Ghouse and Abidi (1979) while studying the effect of different acute doses of gamma-rays on the morphological and quantitative characters such as height and weight of the plants, number of capsules per plant etc., in Var. Neelam of Linum usitatissimum L. observed no marked difference in the height and weight of the plants with 25 and 50 K rad doses which were at par with the controls but with 75 K rad dose they recorded a sudden rise in the number of capsules per plant. Doses higher than 75 K rad proved to be inimical or detrimental for height, weight and number of capsules per plant. Reduction in height with 100, 125 and 150 K rads was 16.9, 18.3 and 23.9 per cent respectively.

PLAN OF STUDY

Morphological study of irradiation effect on some crop plants is proposed to be undertaken according to the following schedule.

1. Conducting pilot experiments to find out effective and efficient doses of X-rays or gamma-rays as well as to ascertain the required period of presoaking on the basis of LD-50.

2. Subjecting the seeds of the selected crop plants to different effective doses of chronic and acute irradiation with X-rays or gamma-rays.

3. Collecting data pertaining to the following parameters.

   (i) Germination response

   (ii) Growth rate

   (iii) Survival of seedlings

   (iv) Morphological variations and abnormalities with respect to:

   (a) Root

   (b) Shoot

   (c) Branching

   (d) Leaf

   (e) Chlorophyll deficiency and distribution

   (f) Flower

   (g) Fruit

(v) Pollen sterility

(vi) Seed sterility

(vii) Yield

4. Statistical analysis

5. Discussion

6. Literature Survey

MATERIALS AND METHODS

1. MATERIALS TO BE STUDIED

Germ plasm of different cultivars and varieties of the following oily and proteinaceous plants will be collected and a selection will be made on the basis of pilot studies:

(i) An oil-yielding variety of sunflower

(ii) Any common cultivar of sesame

(iii) Few varieties of Soybean

(iv) Common varieties of lentil

(v) Some Madhya Pradesh Cultivars of flax.

2 - METHODOLOGY

I. Method of Treatment with Ionizing Radiations (X-rays or gamma-rays)

The seeds presoaked in distilled water for 24 hours arranged in monolayers are to be subjected to acute or chronic irradiation either with gamma-rays from ^{60}Co source or with X-rays. A set of untreated seeds with same moisture content will be used as a control for each treatment. Four replicates of 100 seeds each will be used.

II. Method of Recording Observations

Sensitivity of different oily and proteinaceous seeds preferably towards 10 different doses of radiation will be studied with the help of following parameters.

(1) Germination

Since germination tends to be delayed in the irradiated material, observations on germination of irradiated seeds and their controls are to be recorded every day for as many as 20 days or more, to study the toxic effect of the radiation and period of recovery from the toxicity. Finally the overall germination percentage will be deteriminad. The emergence of coleoptile or radicle will taken as the indication of seed-germination.

(2) Growth Rate

Effect of different treatments on growth rate will be determined in terms of root and shoot elongation and dry weight of 10-day and 20-day old seedlings. For this purpose mean of the data collected for 50 seedlings will be taken. The value of growth in length and dry weight will be expressed in centimeters and miligrams respectively.

(3) Survival of seedlings

The survival percentage will be computed on the basis of values obtained with respect to percentage of plants surviving till maturity out of the total number of plants produced through seed-germination.

(4) Chimeras and other Morphological Abnormalities

In the treated R_1 progenies, appearance of Chlorophyll chimeras and other morphological abnormalities will be screened and carefully recorded and thereafter their frequencies of occurrence will be calculated.

(5) Pollen Sterility

Pollen sterility will be determined from mature anthers of 25 randomly selected plants for each treatment by way of staining the pollen with 2% acetocarmine mixed with equal quantity of glycerine. Pollen grains which fail to take stain or exhibit abnormal shape accompanied with improper filling will be considered sterile. Percentage of occurrence of such sterile

pollen grains will be determined.

(6) Seed Sterility

Ten fruits from each of the 25 randomly selected fully mature R_1 plants per treatment will be studied for seed sterility. From each fruit thin and papery seeds will be sorted out which will be taken as sterile and their percentage of occurrence for each individual fruit will be determined, with the help of which average seed sterility of 25 randomly selected plants will be determined and this will be regarded as sterility due to irradiation.

(7) Statistical Analysis

Data Collected on various characters in R_1 generation is to be analysed according to randomised plot design as has been worked out by Panse and Sukhatme (1967) after calculating the black means. Based on Critical Difference (C.D.) and Standard Error (S.E.) the significance or otherwise of the irradiation effect will be statistically worked out.

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